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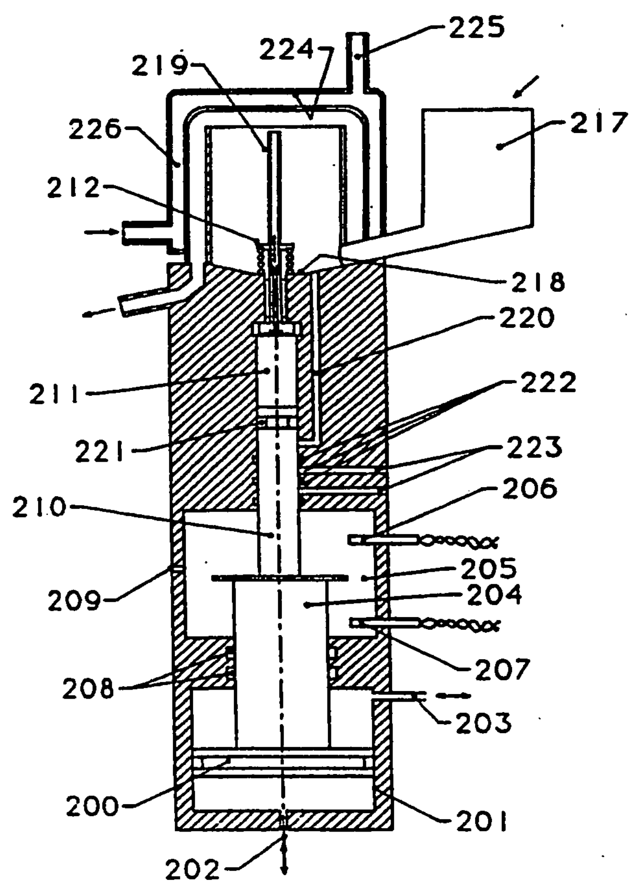
(54) Title: APPARATUS FOR DISRUPTING CELLS

(57) Abstract

Apparatus for disrupting cells comprises a hydraulic system (202, 203) controlled through control means to move a piston (200, 204, 210) to apply pressure to cell culture in a cylinder (211). A very fine restricted orifice (214) is provided at the end of the cylinder (211) opposite the piston (210) and the control means controls the hydraulic system such that a substantially constant pressure is exerted on the cell culture in the cylinder (211) to force the cells through the orifice (214). After the orifice (214) the cells impact on an inpact surface (224, 90, 250, 604).



APPARATUS FOR DISRUPTING CELLS

This invention relates to an apparatus for disrupting cells, for example culture cells. It may be desirable to disrupt culture cell walls in order to extract internal matter from the cells for the extraction of genetic material or chemical compounds contained with the cell.

In known apparatus for this purpose, it has been proposed to force a culture through a relief valve by a mechanically operated piston means, the relief valve opening under the pressure applied to the culture to provide a restricted orifice which varies according to the applied pressure and/or the nature of the culture being passed through the apparatus. It is an object of the present invention to provide a new or improved apparatus which may be capable of more consistent operation at higher pressures when necessary and be suitable for use with cleaning and sterilizing cycles without the need to dismantle the apparatus. Arrangements are also proposed for energy recovery systems.

According to a first aspect of the invention there is provided apparatus for disrupting cells comprising pressure applying means and means defining a

restricted orifice of fixed dimensions, the pressure applying means being arranged to force cells through the orifice.

In the invention, the orifice is of fixed dimensions and no relief valve varying the size of the orifice is provided. Problems associated with maintenance and repair of a relief valve are therefore not present.

Preferably, the pressure applying means includes hydraulic means. This is different from the known apparatus which uses mechanically operated piston means and enables more flexible control pressure application. The hydraulic means is preferably controlled by control means arranged to provide a substantially constant pressure on cells being forced through the orifice. The apparatus can thus be precisely controlled and hence the pressure at the orifice can be controlled for optimum disruption conditions.

According to another aspect of the invention there is provided apparatus for disrupting cells comprising means defining an orifice, hydraulic means for applying pressure to force cells through the orifice and control means arranged to control the hydraulic means to provide a substantially constant pressure on

cells being forced through the orifice.

The means defining the orifice preferably defines an orifice of fixed dimensions.

The pressure applying means is preferably arranged to magnify the pressure from a hydraulic source such that the pressure applied to the cells is greater than the pressure of the source. In this way a relatively low pressure hydraulic source can be utilised. The pressure applying means may conveniently include a piston and the piston may be arranged to reciprocate to provide cell disruption at least semi-continuously.

Preferably, the means defining the orifice comprises a separate removable component. In this way cleaning and repair of the means defining the orifice, if necessary, can be carried out easily with the component removed.

The cells may be introduced into a compression chamber including the orifice through a valve which moves inwardly to open. Preferably, the orifice is defined in the valve. This provides a compact arrangement with fewer regions for leakage from the compression chamber.

The orifice may have a diameter in the range 0.1 to 0.5mm.

An impact surface may be provided downstream of the orifice. The impact surface is preferably cooled and may be cooled by a fluid cooling jacket. In the prior known device utilising a pressure release valve, the cells may be heated by the pressure build in opening the valve or at small valve openings resulting in a potential denaturing or degrading of the chemical to be extracted. By the use of an orifice of fixed dimensions, such heating of the valve is avoided and by cooling of the impact surface it can be ensured that the temperature of the cells does not rise undesirably. The impact surface may be an impact member which is arranged to be moved by the impact of the cells. The impact member may be a piston and in one embodiment the piston is arranged such that movement thereof increases the pressure in the hydraulic means. Alternatively, the impact member may be at least one blade arranged to be rotated by the impact of the cells. The or each blade may be connected to a centrifuge which may be for centrifuging the cells. In another embodiment, means is provided to utilise the jet of cells from the orifice in the manner of a pump. In these ways, the kinetic energy of the cells is not lost, but instead is utilised in the course of processing the cells.

Where the pressure applying means includes a piston, preferably one of the pistons and its cylinder includes a portion of reduced wall thickness, the volume behind the portion being capable of being pressurised by the piston. In this way, the pressure can flex the portion to decrease or eliminate the gap between the piston and cylinder.

According to another aspect of the invention, there is provided a piston and a cylinder, one of the piston and cylinder including a portion of reduced wall thickness, the volume behind the portion being capable of being pressurised by the piston.

Preferably, the portion extends around substantially the entire circumference of the piston or cylinder.

In one embodiment, the piston includes the portion and the volume behind the portion forms a recess in the crown of the piston. In another embodiment, the cylinder includes the portion and the portion comprises a separate component mounted about the piston.

According to a further aspect of the invention, there is provided a shaft in a pressurised chamber, the

shaft journal including a portion of reduced wall thickness to define a volume therebehind which is subject pressure in the chamber.

Hence the principle of the invention is applicable to a shaft in a journal as well as a piston and a cylinder.

The portion preferably comprises a separate component mounted about the shaft.

The portion is preferably arranged to flex under pressure in excess of 10,000 psi.

The apparatus may be operated in single form or a number of units may be integrated together to provide increased capacity.

A number of embodiments of the invention will now be described by way of example and with reference to the accompanying drawings, in which:

Fig. 1 is an axial plane section of one form of the apparatus;

Fig. 2 is a detail axial plane sectional view on an enlarged scale of the fine bore jet and inlet valve shown in Fig. 1;

Fig. 3 is an alternative arrangement of the fine bore jet and inlet valve;

Fig. 4 is an elevation in cross-section of a seal;

Fig. 5 is an elevation in cross-section of another seal;

Fig. 6 is an axial plane section of one form of energy recovery system;

Fig. 7 is a diagram of a turbine energy reclamation unit;

Fig. 8 is an elevation in cross-section of a centrifuge arrangement;

Fig. 9 is a diagram of a cyclone filter arrangement powered by the energy of the jet;

Fig. 10 is a diagram of a membrane filtration unit powered by the energy of the jet, and

Fig. 11 is a diagram of a mixing system using the energy of the jet.

Referring to Figs. 1 and 2 of the drawings showing sections of the apparatus a hydraulic piston 200 is arranged to act in a cylinder 201 in a double acting manner by the supply of hydraulic fluid through inlet/outlet ports 202 and 203. An extension 204 of the piston enters a chamber 205 in which proximity sensors or limit switches 206, 207 are arranged to detect and reverse the travel of the piston 200. Double seals 208 are provided to prevent leakage of

hydraulic fluid into the chamber 205 but any fluid which finds its way into chamber 205 can be vented at 209 to atmosphere.

A small diameter intensifier piston 210 projects from the forward end of the piston 200 to act on the cell culture in a restricted diameter cylinder 211 coaxial with the cylinder 201.

The cell culture passes into the cylinder 211 through a spring loaded non-return valve shown in more detail in Fig. 2. The valve 212 comprises a valve body 213 which includes a very fine restricted orifice 214 through which the cell culture is forced for disruption. The valve body 213 seats at 215 at an end of the cylinder 211 and is spring loaded at 216 into the closed condition.

In a manually loaded version of the apparatus cell culture is introduced through an inlet 217 into a conical receptacle 218 surrounding the inlet valve 212. Arrangements can be made to automatically fill the inlet 217 but this does not affect the operation of the disrupter.

When the hydraulic piston 200 is withdrawn on its intake stroke, the valve body 213 lifts off the

seating 215 against the pressure of the spring 216 and draws the culture from the hopper 218 into the restricted cylinder 211. When the proximity sensor 207 detects the end of the intake stroke, the hydraulic piston 200 is reversed, and the intensifier piston 210 forces the culture against the valve 212 closing it and forcing the culture through the orifice 214 during the delivery stroke.

A passageway 220 provides for any leaked culture material escaping past the high pressure seal 221 of the intensifier piston to be returned to the region of the hopper 218. Triple low-pressure seals 222 and steam purge inlets 223 are provided to allow heated fluid such as steam to be introduced between the seals 222.

The piston 200 is hydraulically operated by a conventional hydraulic pressure supply at pressures up to 200 bar (approx. 3,000 psi) but the pressure applied by the piston 210 to the culture may be of the order of 2750 bar (approx. 40,000 psi) to cause cell wall disruption in the culture. The exact manner in which the cell wall is disrupted is not fully understood and may be caused by the compression together of the cells or more likely by the sudden drop in pressure as the culture passes though the jet

214.

Owing to the manner in which the hydraulic system can be controlled using conventional control techniques high pressure can be rapidly applied to the culture immediately prior to its passage through the jet 214 and can be maintained at an adjustable but constant value throughout the delivery stroke. Therefore the fine bore of the jet 214 can be of simple fixed or non-variable form as compared with the variable orifices provided by the known use of a relief valve. The jet 214 may be made in ruby. The rapid high pressure application and use of a non-variable jet 214 also enables consistent and continuous operation of the apparatus to be obtained by reciprocation of the pistons 200 and 210 as against the "single shot" operation of prior mechanical forms of apparatus.

An alternative form of inlet valve is shown in Fig. 3. The fine restricted orifice 214 is mounted directly in the end of the cylinder 211, an annular valve plate 230 is arranged to have restricted axial movement by the step 233 in the cylinder 211. During the delivery stroke the valve plate 230 seats on the end of the cylinder 211 at 232 sealing off the inlet holes 231, during the inlet stroke the valve plate 230 moves axially as limited by the step 233 uncovering the

holes 231 allowing culture into the cylinder 211.

The disrupted extract is forced in a jet through a tube 219 at high velocity and strikes the interior of a rounded cylindrical container 224. The extract contains a great deal of kinetic energy and spreads in a film across the surface of the container. The friction between the fluid and the inside of the container slows the fluid down converting the kinetic energy to heat which if allowed to increase the temperature of the extract could damage or denature it. A coolant such as chilled water or liquid nitrogen is fed at 225 into a coolant jacket 226 surrounding the container 224 which acts as a thin walled heat exchanger.

Fig. 4 is a detail view of the piston 210 and shows the high pressure seal 221 which comprises a rubber "O" ring 300 with a further ring 301 radially outside it. The inner ring 300 may be made from rubber and the outer ring may be made from PTFE.

The rings 300, 301 and are able to provide a compact sealing arrangement capable of working at pressures up to 20,000 psi provided attention is given to finish of the cylinder wall 302 in contact with the ring 301 and the radial clearance "c" between the cylinder wall 302

and the piston 211. In the context of sealing this clearance is known as the extrusion gap.

The piston includes a piston head 303.

The clearance "C" between the piston 211 and the cylinder wall 302 is such that there is no possibility of contact between those components. Such clearance is say typically 0.010" radially and enables the materials of the cylinder wall 302 and the piston head 303 to be chosen without consideration to their ability to run together with close clearance.

The piston head 303 is attached to the operating piston 211 either by mechanically snapping over the protrusion 304 which is an integral part of the piston 211 or when the choice of material for component 303 permits by being moulded onto the protrusion 204. The value of the extrusion gap "c" as manufactured is chosen to be as small as possible consistent with ease of assembly, typically 0.0005". The piston head 303 is detailed so that its length is typically but not necessarily 1.5 times its diameter and may be provided with a series of labyrinth grooves 305. The material for the piston head 303 is chosen so as to be able to operate in close contact with the cylinder wall 302 either statically or in a reciprocating fashion as

necessary.

The crown 306 of the piston head 303 is hollowed over to provide a portion 307 which is relatively thin walled around the circumference of the piston.

In operation at low pressures the piston head 303, the inner rubber "O" ring 300 and the outer ring 301 work a "standard" sealing arrangement able to operate with the extrusion gap "c" as set by the machining tolerances. As the pressure increases the effect of fluid pressure in the hollowed out end in piston head 303 is to expand the portion 307 into intimate contact with the cylinder wall 302 reducing the extrusion gap "c" to a value approaching zero. Further increases in pressure expand the whole portion 307 into intimate contact with the cylinder wall 302 due to the high axial loads involved reducing the extrusion gap to zero.

In the alternative arrangement shown in Fig. 5 a seal housing 310 is snapped or moulded into a groove 311 in the cylinder wall 302 to seal against the piston shaft 211. The seal housing 310 performs the same function as the portion 307 in Fig. 4 and the same criteria with respect to choice of material apply. Low pressure sealing is achieved by the action of the

inner ring 300 and outer ring 301 as already described. As the pressure increases the effect of the fluid pressure in chamber 14 is to compress the component 310 reducing the extrusion gap "c" to a value approaching zero. Further increases in pressure expand the whole seal housing into intimate contact with the piston 211 due to the high axial loads involved reducing the extrusion gap "c" to zero.

In the embodiment of Fig. 1 the disrupted cell extract is passed direct to the heat exchanger where the energy introduced to enable the disruption of the cells is extracted without allowing the extract to increase in temperature but with the penalty that this energy is lost. This loss of energy may not be important in small scale laboratory situations but in the case of larger scale installations operating continuously the loss of energy is of great importance as the energy could be utilised.

Figs. 6 to 11 are proposals for extracting energy from the jet and using the energy to power various aspects of the overall process.

In the development shown in Fig. 6 which replaces the cylindrical container 224 and the coolant jacket 226 the high velocity jet of culture extract issuing from

the fine bore of the jet 214 into a passageway 81 is directed at a recovery jet 8 which opens out into chamber 80 where the kinetic energy of the jet is converted to pressure which acts upon the small diameter piston 90 connected to or acting on a large diameter piston 91 whereby the latter is thrust back against its loading spring 93 to expel fluid under pressure into the operating hydraulic circuit at 92 and thus augment the pressure operation thereof.

On return movement of the piston 91 the piston 90 is returned to cause its head to readily return culture extract via a relief valve 94 to the delivery outlet duct 510 rather than back through the recovery jet 80. The relief valve member 95 is shown in the form of a piston which is urged to the closed position by hydraulic fluid pressure.

In the development shown in Fig. 7 the kinetic energy of the jets associated with the disrupter units 215 and 252 is directed onto a turbine 250 which is able to convert the energy to mechanical energy in rotational form available for use in driving machinery such as centrifuges.

Fig. 8 shows a centrifuge 600 comprising a cylindrical casing 601 having a central shaft 602

rotatably mounting a centrifuge drum 603. The drum 603 includes blading 604 on its upper surface and has apertures leading into the drum 603. A duct 605 leads from the jet 214 to the centrifuge 600 and directs the jet of cells at an acute angle to the top surface of the drum 603 along a chord thereof such that cells from the duct 605 striking the drum 603 act to drive the drum 603. Cells that have hit the upper surface of the drum fall through the apertures in the top surface and the cell walls and other relatively heavy material is centrifuged outwardly. Desired material will fall through appropriately positioned apertures 607 in the base of the drum 603 and then through bore 606 in the base of the centrifuge for collection.

Fig. 9 shows a cyclone separation unit and Fig. 10 showing a membrane filtration unit are examples of conventional equipment normally involving the use of a pump able to be driven by the energy in the disrupter jet.

Fig. 11 shows a development using the energy of the jet from a disrupter unit 260 to power a venturi mixing/reaction device 262 enabling the output from a disrupter unit to draw reaction fluid from a container 263 to be mixed with the process fluid without the use of additional pumping and mixing equipment.

Single disruption units have been built operating on a cyclic basis and higher throughputs can be achieved by manifolding a multiplicity of units into one high flow unit.

CLAIMS

1. Apparatus for disrupting cells comprising pressure applying means and means defining a restricted orifice of fixed dimensions, the pressure applying means being arranged to force cells through the orifice.

2. Apparatus as claimed in claim 1, wherein the pressure applying means is operated by hydraulic means.

3. Apparatus as claimed in claim 2, wherein the hydraulic means is controlled by control means arranged to provide a substantially constant pressure on cells being forced through the orifice.

4. Apparatus for disrupting cells comprising means defining an orifice, hydraulic means for applying pressure to force cells through the orifice and control means arranged to control the hydraulic means to provide a substantially constant pressure on cells being forced through the orifice.

5. Apparatus as claimed in claim 4, wherein the means defining the orifice defines an orifice of fixed dimensions.

6. Apparatus as claimed in any of claims 2 to 5, wherein the pressure applying means is arranged to magnify the pressure from a hydraulic source such that the pressure applied to the cells is greater than the pressure of the source.

7. Apparatus as claimed in any preceding claim, wherein the pressure applying means includes a piston.

8. Apparatus as claimed in claim 7, wherein the piston is arranged to reciprocate to provide cell disruption at least semi-continuously.

9. Apparatus as claimed in any preceding claim, wherein the means defining the orifice comprises a separate removable component.

10. Apparatus as claimed in any preceding claim, wherein the cells are introduced into a compression chamber including the orifice through a valve which moves inwardly to open.

11. Apparatus as claimed in claim 10, wherein the orifice is defined in the valve.

12. Apparatus as claimed in any preceding claim, wherein the orifice has a diameter in the range 0.1 to

0.5mm.

13. Apparatus as claimed in any preceding claim, wherein an impact surface is provided downstream of the orifice.

14. Apparatus as claimed in claim 13, wherein the impact surface is cooled.

15. Apparatus as claimed in claim 14, wherein the impact surface is cooled by a fluid cooling jacket.

16. Apparatus as claimed in claims 13, 14 or 15, wherein the impact surface is on an impact member which is arranged to be moved by the impact of the cells.

17. Apparatus as claimed in claim 16, wherein the impact member is a piston.

18. Apparatus as claimed in claim 7, or any claim dependent thereon and where hydraulic means is provided, wherein the piston is arranged such that movement thereof increases the pressure in the hydraulic means.

19. Apparatus as claimed in claim 16, wherein the

impact member is at least one blade arranged to be rotated by the impact of the cells.

20. Apparatus as claimed in claim 19, wherein the or each blade is connected to a centrifuge.

21. Apparatus as claimed in any preceding claim, wherein means is provided to utilise the jet of cells in the orifice in the manner of a pump.

22. Apparatus as claimed in claim 7, or in any subsequent claim dependent thereon, wherein one of the pistons and its cylinder includes a portion of reduced wall thickness, the volume behind the portion being capable of being pressurised by the piston.

23. A piston in a cylinder, one of the piston and cylinder including a portion of reduced wall thickness, the volume behind the portion being capable of being pressurised by the piston.

24. Apparatus as claimed in claim 22, or a piston and a cylinder as claimed in claim 23, wherein the portion extends around substantially the entire circumference of the piston or cylinder.

25. Apparatus as claimed in claim 22 or 24, or a

piston and a cylinder as claimed in claim 23 or 24, wherein the piston includes the portion and the volume behind the portion forms a recess in the crown of the piston.

26. Apparatus as claimed in claim 22 or 24, or a piston and a cylinder as claimed in claim 23 or 24, wherein the cylinder includes the portion and the portion comprises a separate component mounted about the piston.

27. A shaft in a pressure chamber, the shaft journal including a portion of reduced wall thickness to define a volume therebehind which is subject to pressure in the chamber.

28. A shaft in a pressure chamber as claimed in claim 27, wherein the portion comprises a separate component mounted about the shaft.

29. Apparatus as claimed in any of claims 22 to 26, or a piston and a cylinder as claimed in any of claims 23 to 26, or a shaft in a pressure chamber as claimed in claim 27 or 28, wherein the said portion is arranged to flex under pressure in excess of 10,000 psi.

30. Apparatus as claimed in claim 10 or any subsequent claim dependent thereon, wherein the valve is a poppet valve.

31. Apparatus as claimed in claim 10 or any subsequent claim dependent thereon, wherein the valve comprises a freely movable ring covering input apertures.

AMENDED CLAIMS

[received by the International Bureau on 11 August 1992 (11.08.92); original claims 1-5 replaced by amended claim 1; claims 6-31 unchanged but renumbered as claims 2-27 (5 pages)]

1. Apparatus for disrupting cells comprising means defining an orifice of fixed dimensions, hydraulic means for applying pressure to force cells through the orifice and control means arranged to control the hydraulic means to provide a substantially constant pressure on cells being forced through the orifice.

2. Apparatus as claimed in claim 1, wherein the pressure applying means is arranged to magnify the pressure from a hydraulic source such that the pressure applied to the cells is greater than the pressure of the source.

3. Apparatus as claimed in claim 1 or claim 2, wherein the pressure applying means includes a piston.

4. Apparatus as claimed in claim 3, wherein the piston is arranged to reciprocate to provide cell disruption at least semi-continuously.

5. Apparatus as claimed in any preceding claim, wherein the means defining the orifice comprises a separate removable component.

6. Apparatus as claimed in any preceding claim,

wherein the cells are introduced into a compression chamber including the orifice through a valve which moves inwardly to open.

7. Apparatus as claimed in claim 6, wherein the orifice is defined in the valve.

8. Apparatus as claimed in any preceding claim, wherein the orifice has a diameter in the range 0.1 to 0.5mm.

9. Apparatus as claimed in any preceding claim, wherein an impact surface is provided downstream of the orifice.

10. Apparatus as claimed in claim 9, wherein the impact surface is cooled.

11. Apparatus as claimed in claim 10, wherein the impact surface is cooled by a fluid cooling jacket.

12. Apparatus as claimed in claims 9, 10 or 11, wherein the impact surface is on an impact member which is arranged to be moved by the impact of the cells.

13. Apparatus as claimed in claim 12, wherein the

impact member is a piston.

14. Apparatus as claimed in claim 3, or any claim dependent thereon, wherein the piston is arranged such that movement thereof increases the pressure in the hydraulic means.

15. Apparatus as claimed in claim 12, wherein the impact member is at least one blade arranged to be rotated by the impact of the cells.

16. Apparatus as claimed in claim 15, wherein the or each blade is connected to a centrifuge.

17. Apparatus as claimed in any preceding claim, wherein means is provided to utilise the jet of cells in the orifice in the manner of a pump.

18. Apparatus as claimed in claim 3, or in any subsequent claim dependent thereon, wherein one of the pistons and its cylinder includes a portion of reduced wall thickness, the volume behind the portion being capable of being pressurised by the piston.

19. A piston in a cylinder, one of the piston and cylinder including a portion of reduced wall thickness, the volume behind the portion being capable

of being pressurised by the piston.

20. Apparatus as claimed in claim 18, or a piston and a cylinder as claimed in claim 19, wherein the portion extends around substantially the entire circumference of the piston or cylinder.

21. Apparatus as claimed in claim 18 or 20, or a piston and a cylinder as claimed in claim 19 or 20, wherein the piston includes the portion and the volume behind the portion forms a recess in the crown of the piston.

22. Apparatus as claimed in claim 18 or 20, or a piston and a cylinder as claimed in claim 19 or 20, wherein the cylinder includes the portion and the portion comprises a separate component mounted about the piston.

23. A shaft in a pressure chamber, the shaft journal including a portion of reduced wall thickness to define a volume therebehind which is subject to pressure in the chamber.

24. A shaft in a pressure chamber as claimed in claim 23, wherein the portion comprises a separate component mounted about the shaft.

25. Apparatus as claimed in any of claims 18 to 22, or a piston and a cylinder as claimed in any of claims 19 to 22, or a shaft in a pressure chamber as claimed in claim 23 or 24, wherein the said portion is arranged to flex under pressure in excess of 10,000 psi.

26. Apparatus as claimed in claim 6 or any subsequent claim dependent thereon, wherein the valve is a poppet valve.

27. Apparatus as claimed in claim 6 or any subsequent claim dependent thereon, wherein the valve comprises a freely movable ring covering input apertures.

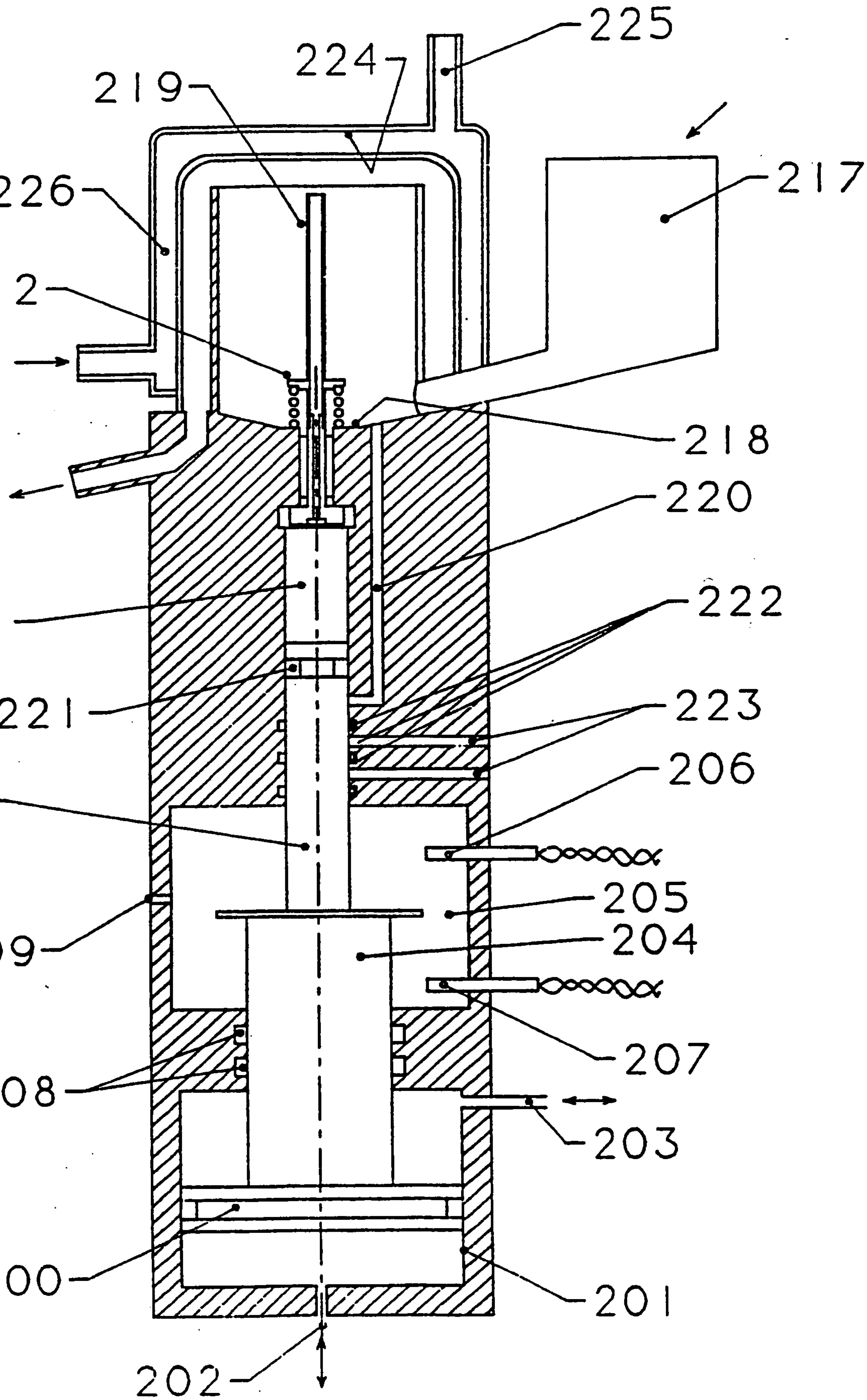

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Fig.1

Fig.2

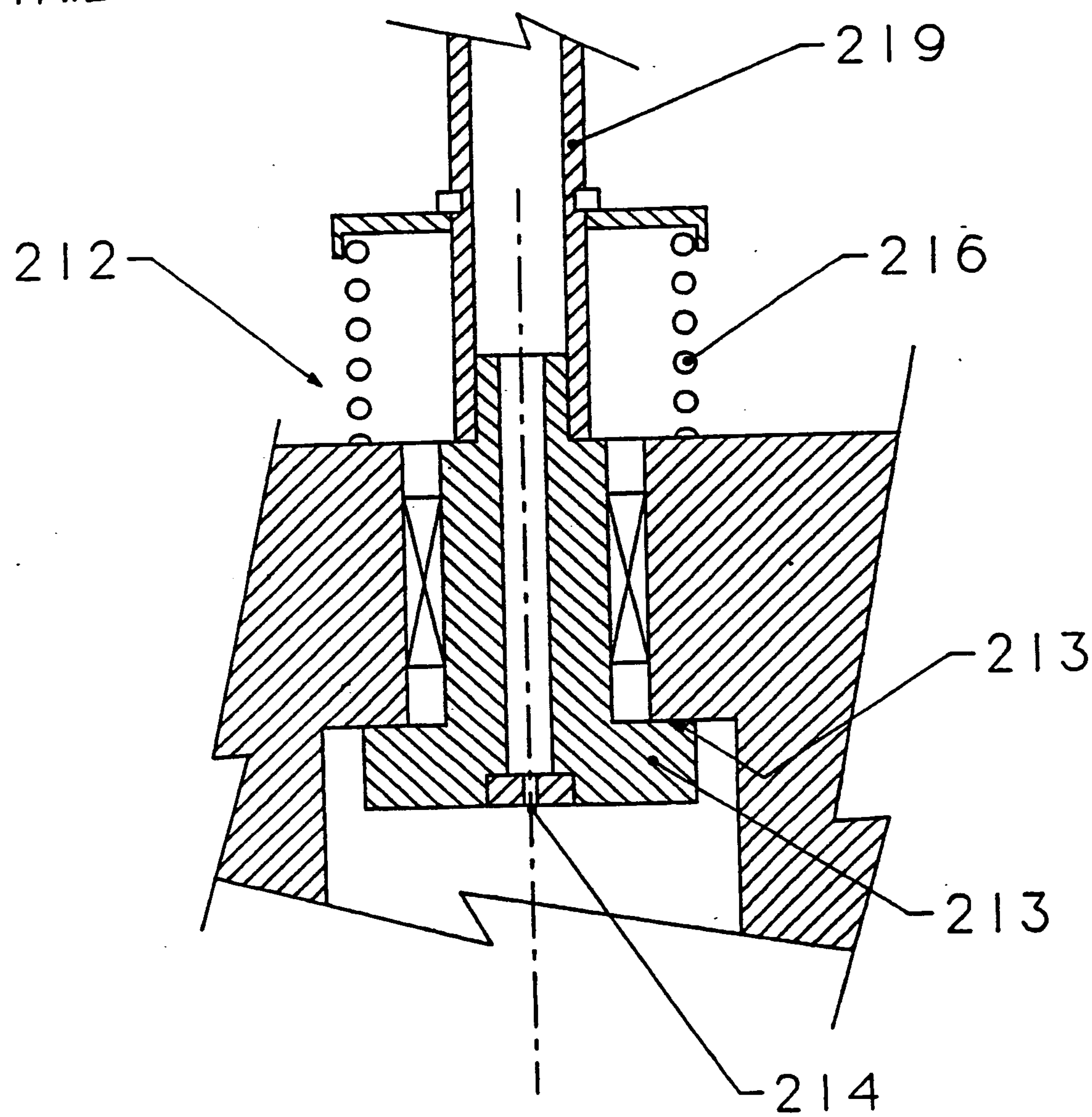

Fig. 3

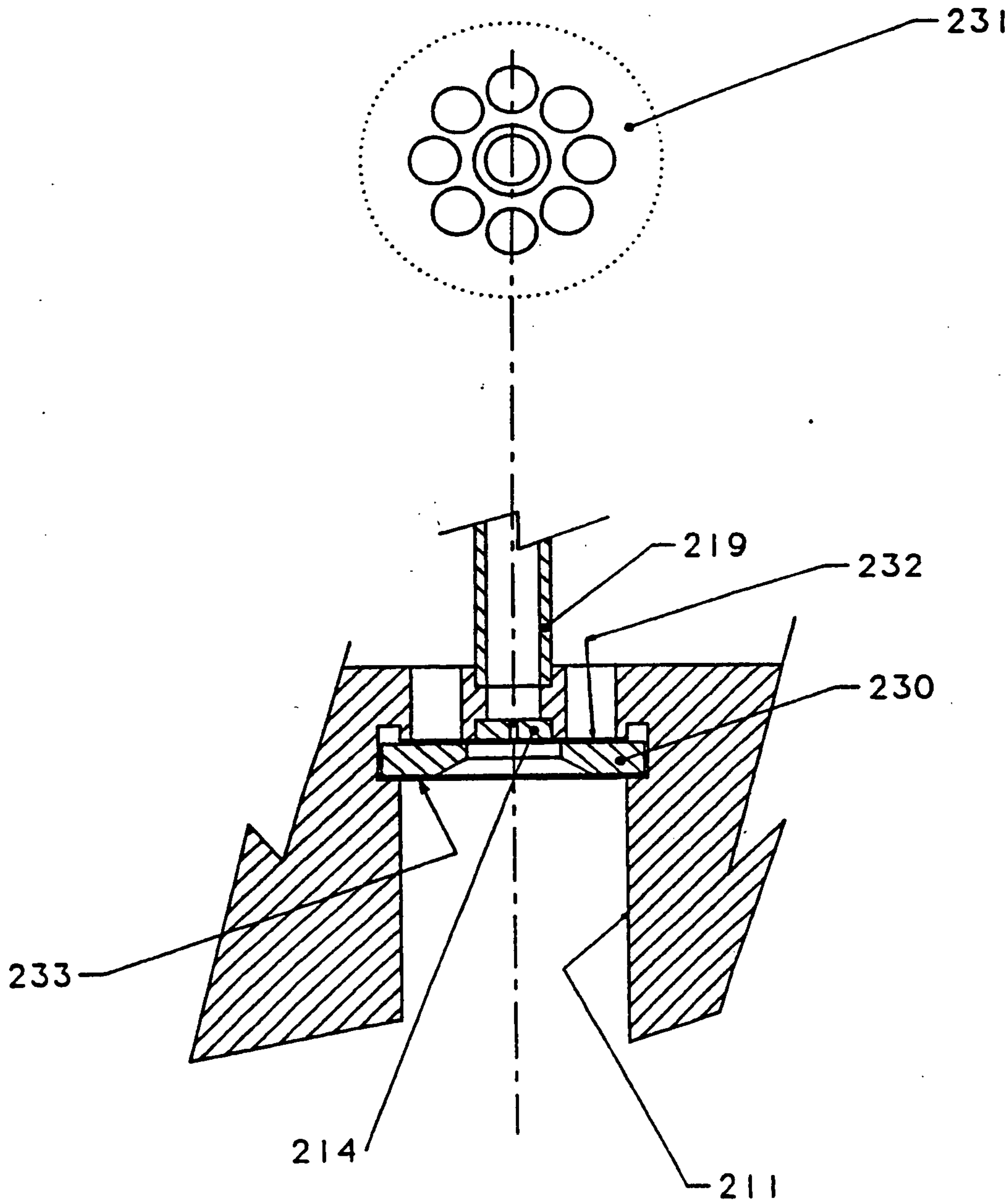

Fig. 4

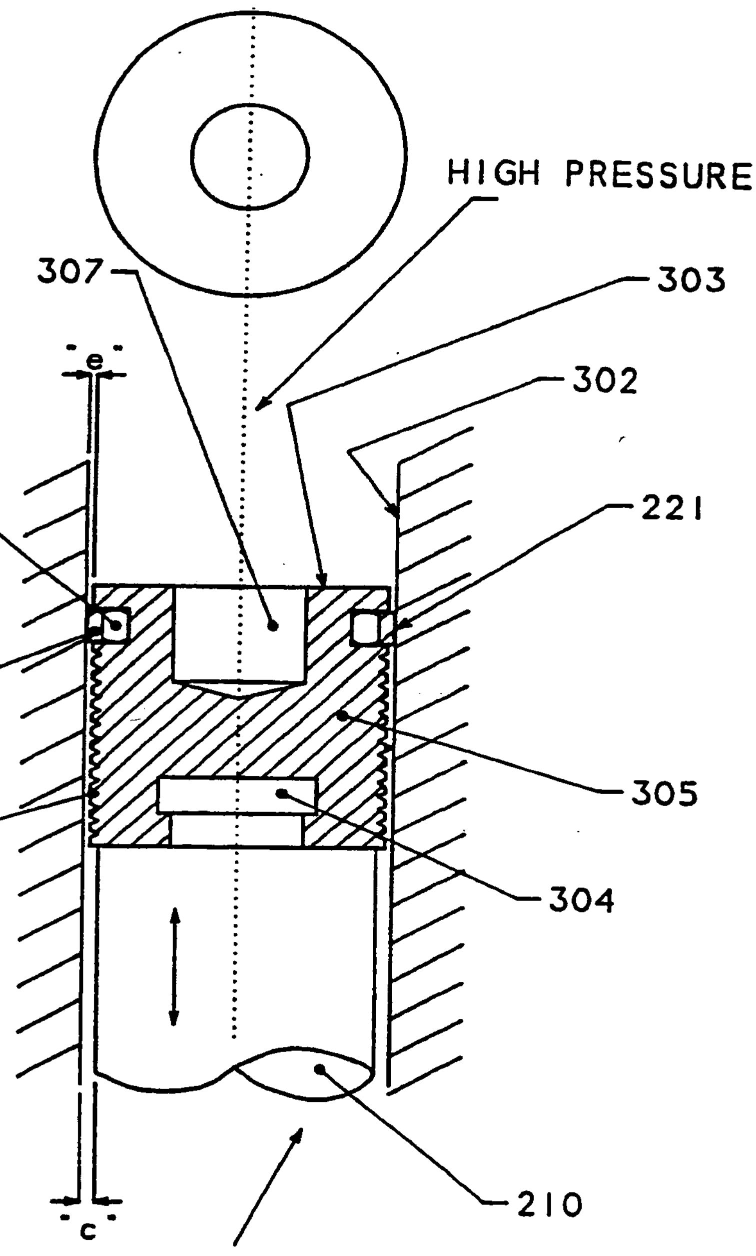
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Fig.5

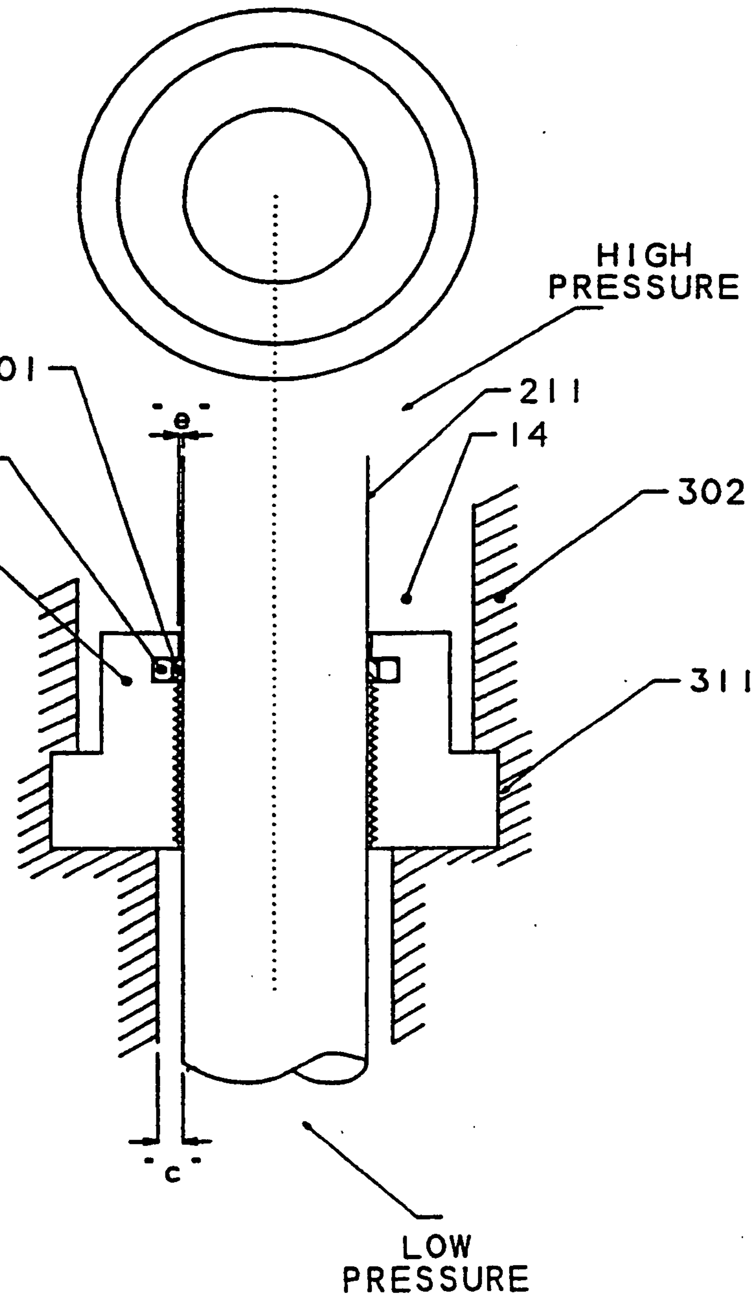

Fig. 6

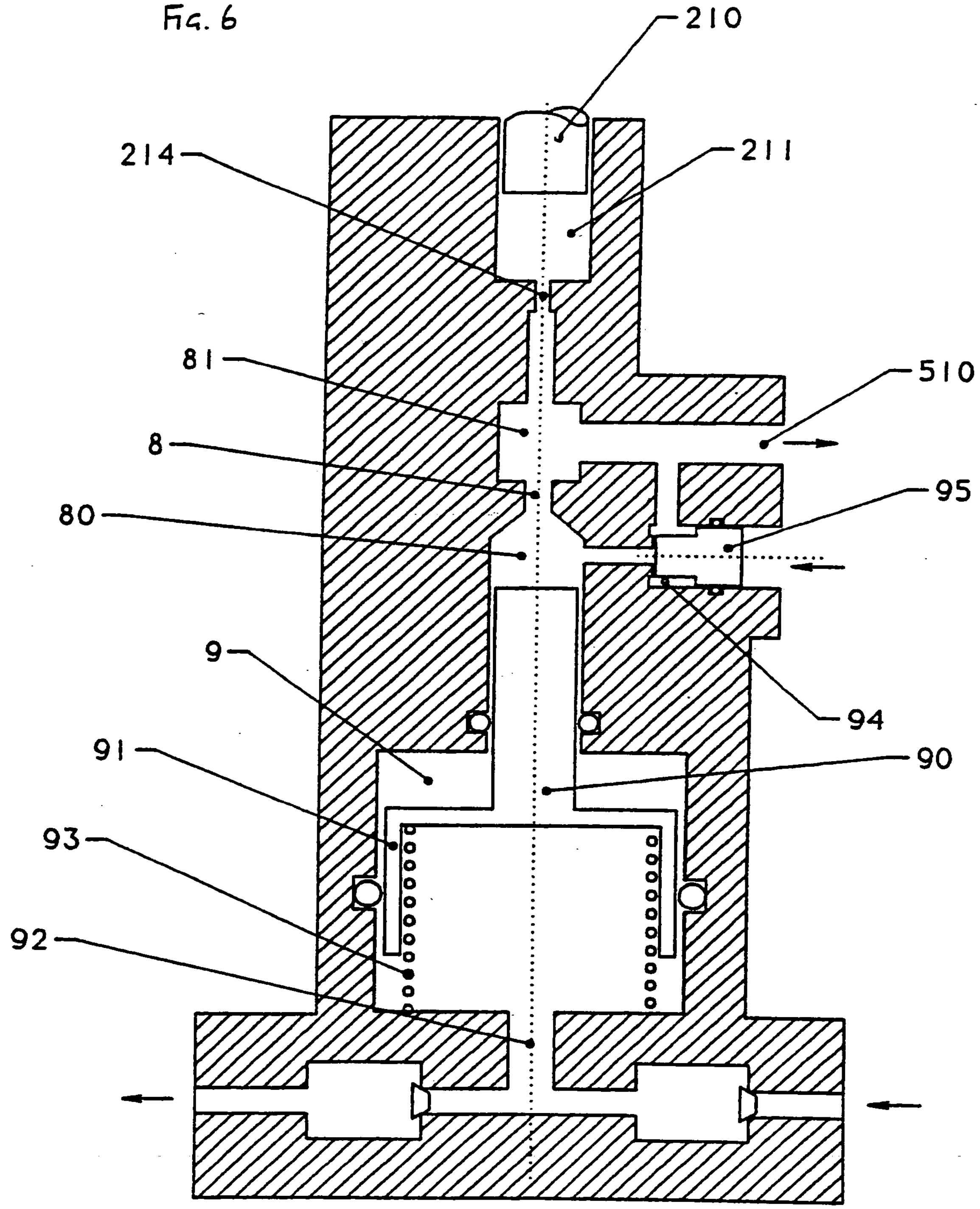

Fig. 7

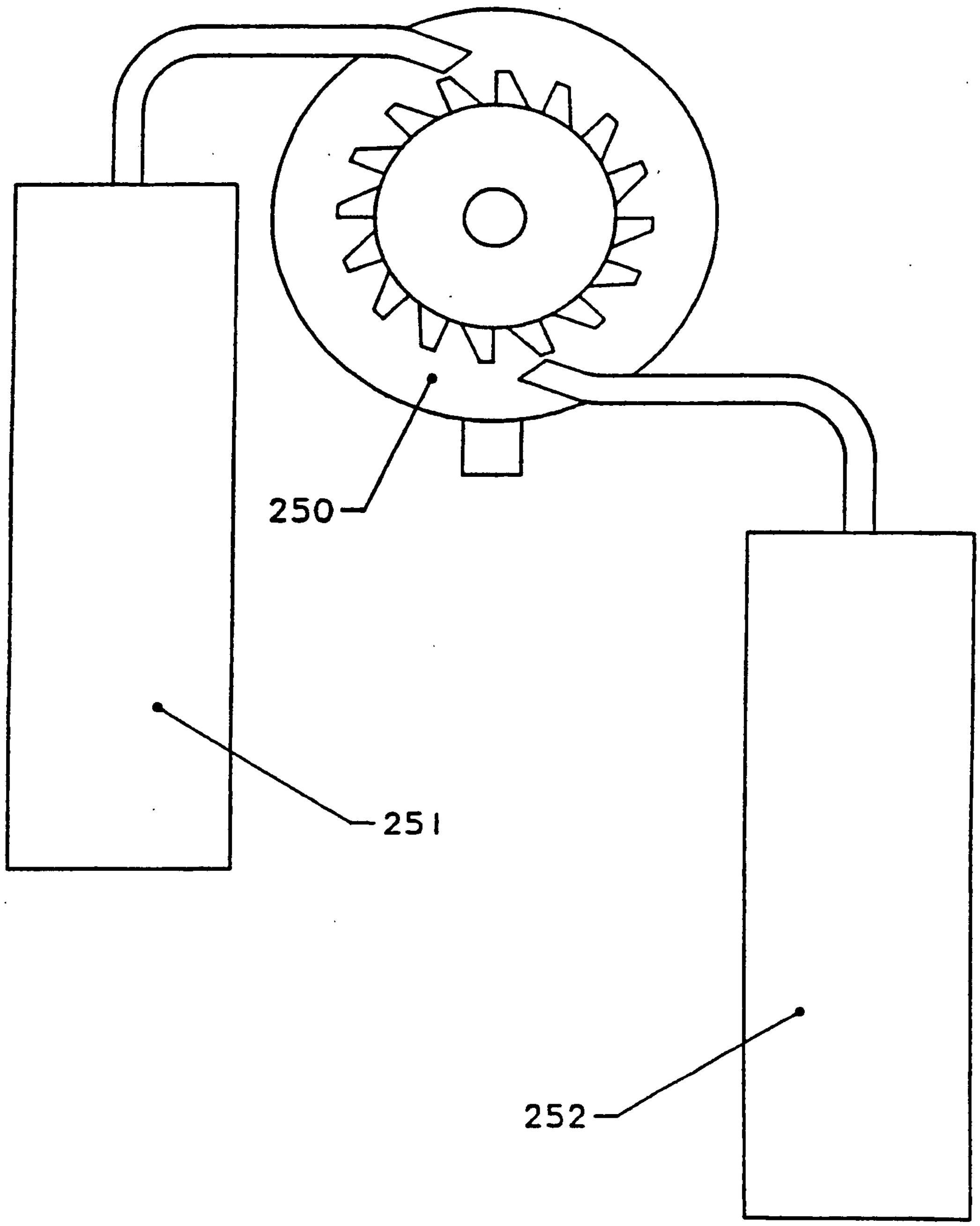

Fig. 8

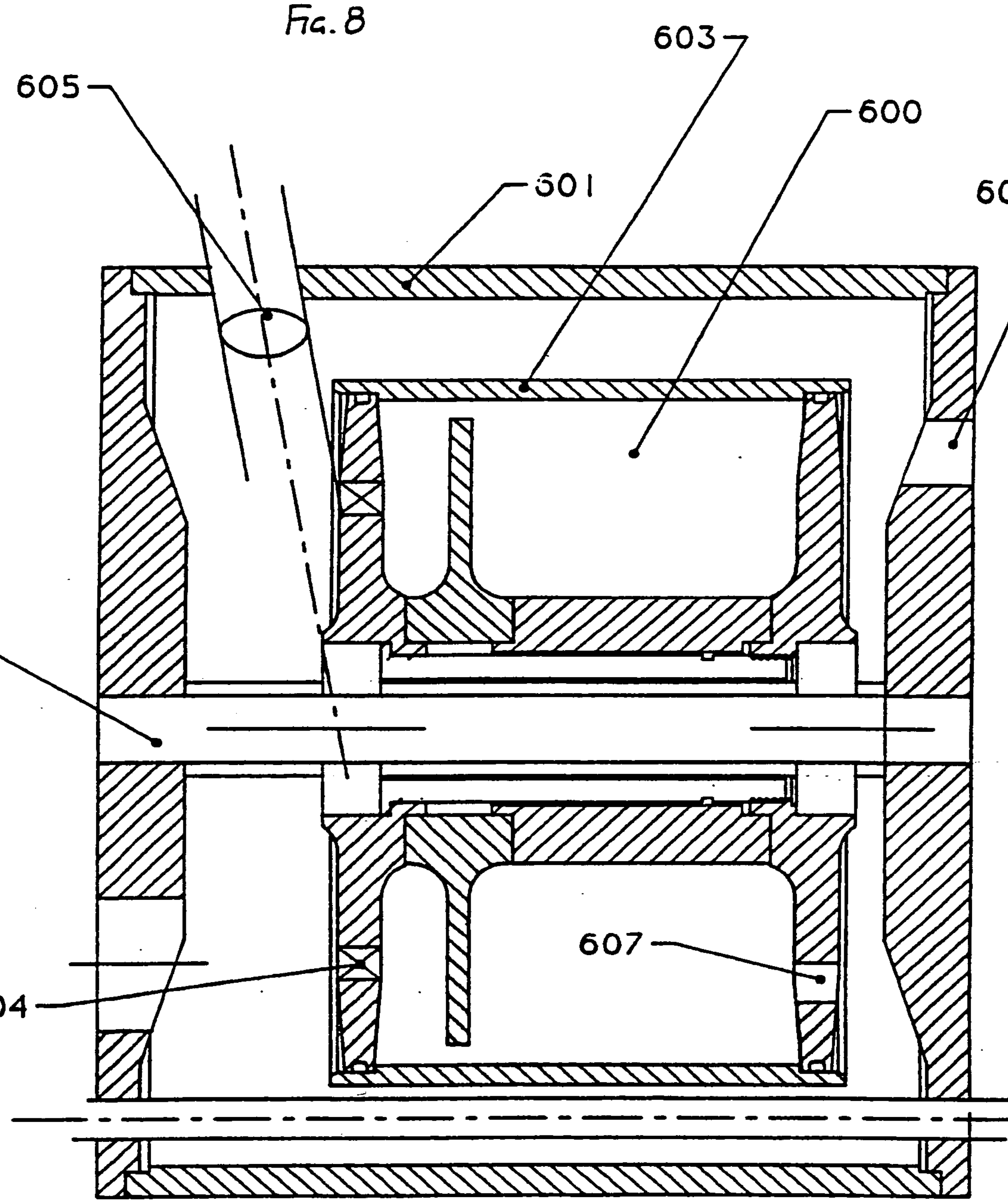

Fig.9

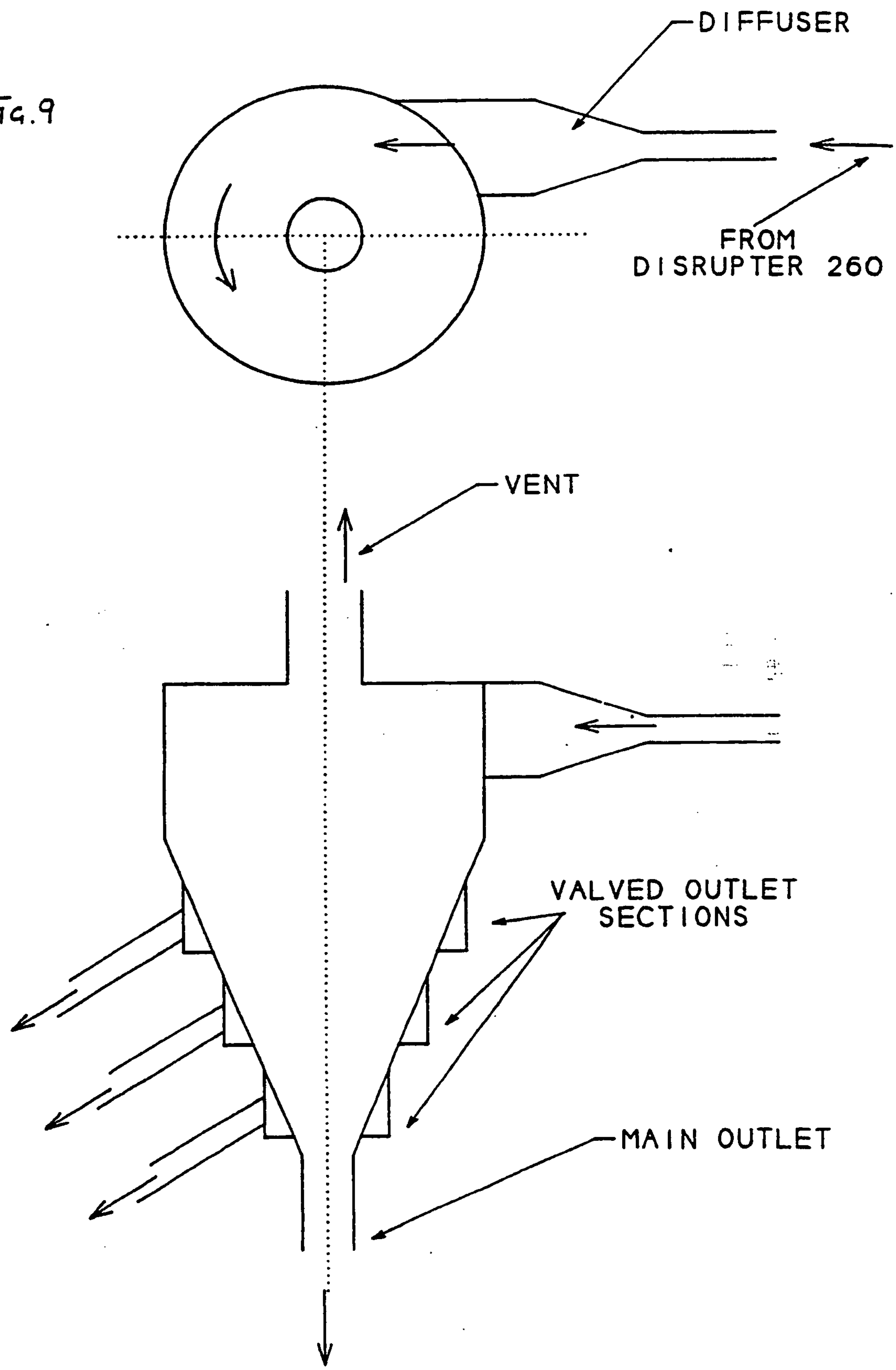
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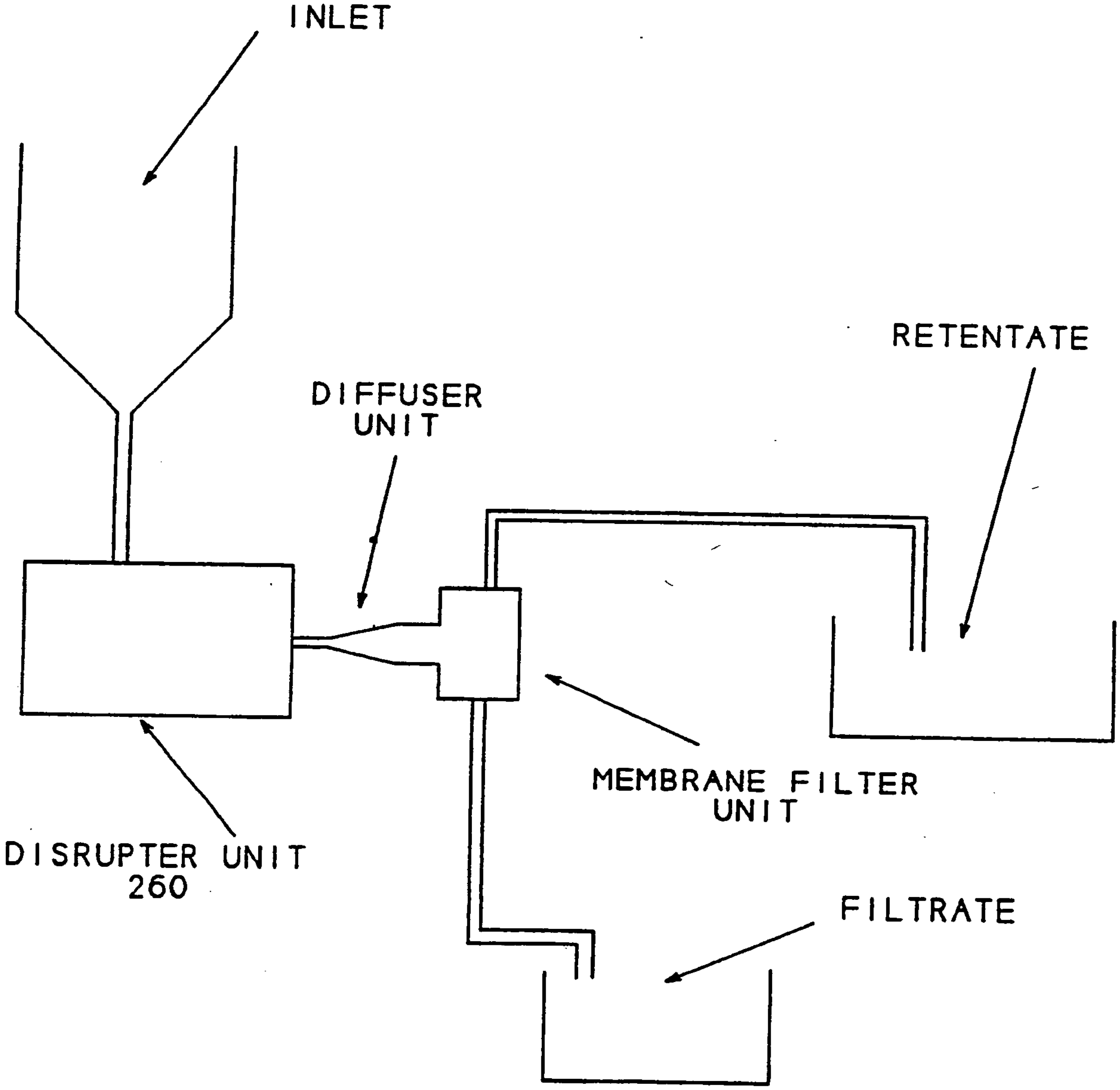
Fig.10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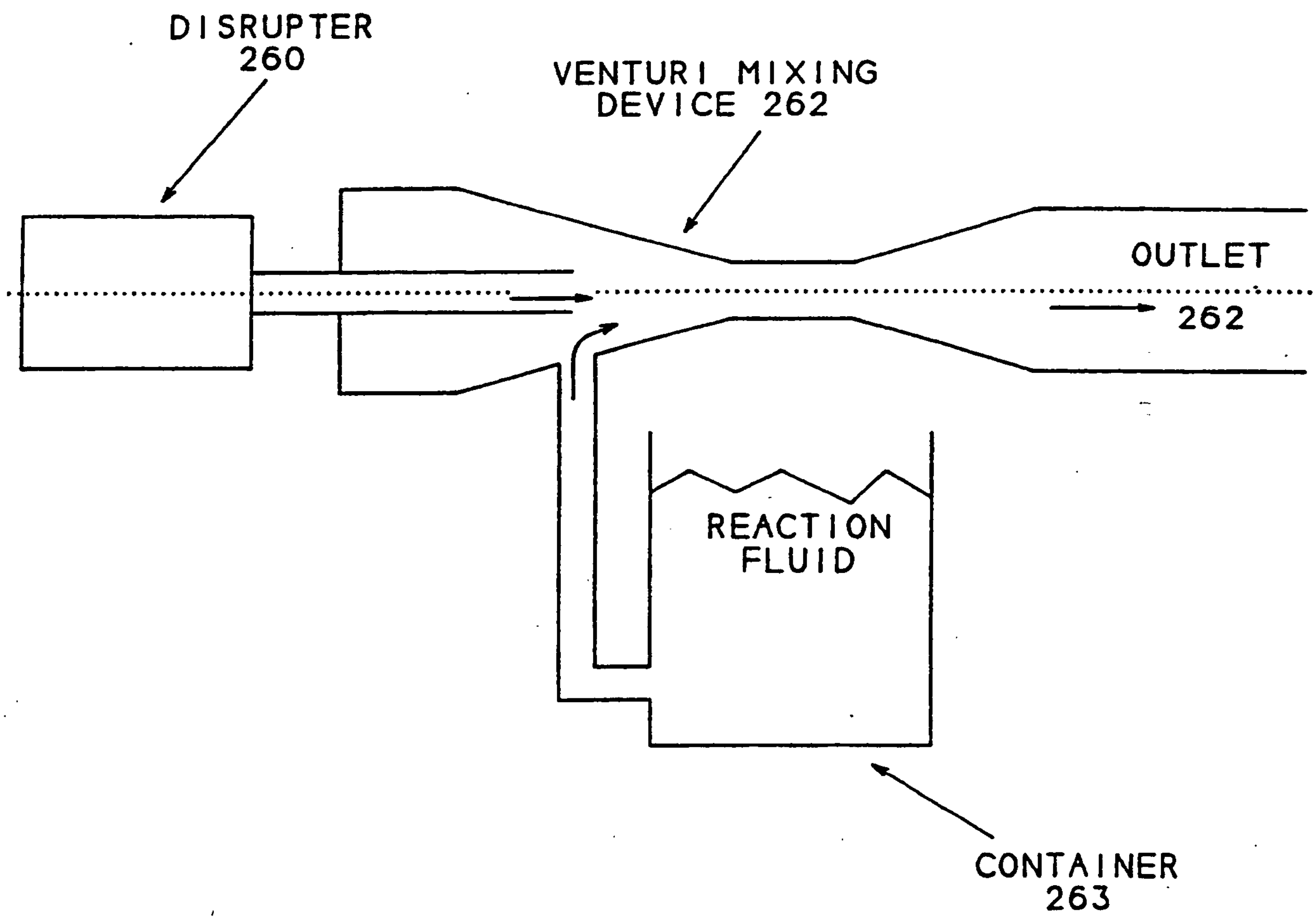
260
FILTRATE

Fig. 11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INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/00421

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12M3/00; B02C19/00; B02C19/12

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12M ; B02C

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	US,A,4 053 110 (S. SCHALKOWSKY ET AL.) 11 October 1977 see column 2, line 53 - column 4, line 52	1,5,7-9, 12-13
Y		3,10-11
X	US,A,4 084 757 (V.J. RAKITIN ET AL.) 18 April 1978 see the whole document	1
Y		3,10-11
X	BIOTECHNOLOGY AND BIOENGINEERING. vol. 23, 1981, NEW YORK US pages 765 - 780; C.R. ENGLER & C.W. ROBINSON: 'Disruption of Candida utilis cells in high pressure flow devices.' see page 767, last paragraph - page 768; figure 1	1
A		13-15

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° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29 MAY 1992	[illegible]
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	BEVAN S.R. SBevan.

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Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	DE,B,1 212 248 (BEHRINGWERKE AKTIENGESELLSCHAFT) 10 March 1966 see the whole document ---	7-8, 16-17, 19-22

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9200421 SA 57231

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 29/05/92

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4053110	11-10-77	None	
US-A-4084757	18-04-78	None	
DE-B-1212248		None	

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82